A promoter is a nucleotide sequence required to facilitate transcription from a transcriptional start site, which is the site at which the first nucleotide of the transcript is transcribed, the nucleotide being complementary to the corresponding nucleotide in the nucleic acid. A promoter operably linked to a transcriptional start site means that the promoter is capable of driving transcription from the transcriptional start site in the absence of further nucleotide sequences.

An enhancer is a nucleic acid sequence which increases the level of transcription from a promoter. Enhancers need not be in any specified position in the nucleic acid in relation to the promoter, transcriptional start site, or transcriptional termination site. All that is required for a specific enhancer to be operably linked to a specific promoter is that the presence of the enhancer increases transcription driven by that promoter.

A transcriptional termination signal is a nucleic acid sequence which terminates transcription of a transcript. A variety of promoters, enhancers, and transcriptional termination signals are known in the art.

A viral expression vector is any combination of a nucleic acid and at least one protein which is useful for delivering a nucleic acid into a cell so as to express a transcript encoded by the nucleic acid in the cell. Other components, such as a lipid bilayer can also be present in the vector. An example of a viral expression vector is a retrovirus.

The invention also includes a transgenic animal (e.g., a mouse or other rodent, pig, rat, cow, chicken, turkey, or sheep) whose somatic and germ line cells contain at least one copy of a transgene comprising (1) a transcriptional start site; (2) a promoter (e.g., a tissue-

specific promoter such as a ζ-globin promoter) operably linked to the open reading frame; and (3) an enhancer operably linked to the promoter. The enhancer includes the nucleotide sequence of SEQ ID NO:1 (e.g., SEQ ID NO:2). The transgenic animal expresses a transcript driven by the promoter, where the level of expression in at least one cell type (e.g., a erythroblast) of the animal is proportionally dependent on the copy number of the transgene, i.e., the greater the copy number, the greater the expression. Such a transcript can be a mRNA encoding a polypeptide (e.g., a growth hormone). In other embodiments, the somatic and germ line cells contain more than 5 copies (e.g., more than 15 copies) of the transgene.

The invention also features a method of expressing a transcript in an animal (e.g., a mouse, pig, rat, cow, chicken, turkey, or sheep) by administering to the animal a nucleic acid comprising (1) an transcriptional start site for the transcript; (2) a promoter (e.g., a tissue-specific promoter such as a ζ-globin promoter) operably linked to the transcriptional start site; and (3) an enhancer operably linked to the promoter, the enhancer comprising the DNA sequence of SEQ ID NO:1 or 2 or the RNA equivalent thereof. The transcript can be a mRNA encoding a polypeptide. The nucleic acid can be administered by parenteral injection (e.g., intramuscular injection) or via a viral expression vector. The nucleic acid can further include a transcriptional termination signal (e.g., a polyadenylation signal).

Nucleic acids and viral vectors containing an enhancer having the mtNF-E2/AP1 sequence described above can be used to express a therapeutic antisense RNA or mRNA encoding a therapeutic polypeptide in an animal in a position-independent and transgene copy number dependent

manner. This was an unexpected result because, previously, transgene expression was limited by position-effect variegation, silencing of transgenes, and the inability to increase expression by increasing the copy number of the transgene. See, e.g., Sabl et al., Genetics 142:447-458, 1996; Palmer et al., Sharpe et al., EMBO J 11:4565-4572, 1992; and Chen et al., Proc Natl Acad Sci USA 94:5798-5803, 1997. By inclusion of an enhancer containing the mtNF-E2/AP1 sequence in the transgene sequence, these deficiencies in transgene expression are removed. Enhancement of transgene expression can result in transgenic animal models exhibiting more severe symptoms so that therapeutic efficacy in those models can be measured in a wider range of symptom severity. Examples of such models, which can be improved by the present invention, are described in U.S. Patent Nos. 5,811,634 and 5,675,060

Other features or advantages of the present invention will be apparent from the following detailed description and also from the claims.

Detailed Description

The invention relates to nucleic acids and viral vectors containing an enhancer with a mutated NF-E2/AP1 site (e.g., the mtHS-40 enhancer), and their use in expressing RNA in an animal. Nucleic acids including the mtNF-E2/AP1 site can be used to form transgenic animals of the invention which express an antisense transcript or a mRNA encoding the protein to be expressed in the transgenic animal. The expression of the transgene is not affected by its position in the genome, nor is the expression inhibited at high transgene copy numbers (e.g., above 5, 7, 9, 14, or 19 copies). Instead, the expression level is directly